AMENDMENTS TO THE CLAIMS:

The following is the status of the claims of the above-captioned application, as amended.

Claim 1. (Currently amended) A 2µm-family plasmid comprising a polynucleotide sequence insertion, deletion and/or substitution between ~~the~~ a first base after ~~the~~ a last functional codon of at least one of either ~~a~~ an *REP* 2 gene or an *FLP* gene and ~~the~~ a last base before ~~the~~ an FRT site in an inverted repeat adjacent to said gene.

Claim 2. (Currently amended) The 2µm-family plasmid of Claim 1 wherein, other than the polynucleotide sequence insertion, deletion and/or substitution, the *FLP* gene and/or the *REP2* gene has the sequence of ~~a~~ an *FLP* gene and/or ~~a~~ an *REP2* gene~~, respectively, derived~~ from a naturally occurring 2µm-family plasmid.

Claim 3. (Currently amended) The 2µm-family plasmid of Claim 1, wherein the ~~naturally occurring 2µm-family~~ plasmid ~~is selected from~~comprises pSR1, pSB3 or pSB4 ~~as obtained~~ from *Zygosaccharomyces rouxii,* pSB 1 from *Zygosaccharomyces baill,,* ~~or~~ pSB2 ~~both as obtained~~ from *Zygosaccharomyces bailli,* pSM1~~as obtained~~ from *Zygosaccharomyces fermentati,* pKD1 ~~as obtained~~ from *Kluyveromyces drosophilarum,* pPM1 ~~as obtained~~ from *Pichia membranaefaciens,* ~~and~~ or the 2µm plasmid ~~as obtained~~ from *Saccharomyces cerevisiae.*

Claim 4. (Currently amended) The 2µm-family plasmid of Claim 2 wherein the sequence of the inverted repeat adjacent to said *FLP* and/or *REP2* gene is ~~derived~~ from the sequence of the corresponding inverted repeat in the same naturally occurring 2µm-family plasmid as the sequence from which the gene is ~~derived~~from.

Claim 5. (Currently amended) The 2µm-family plasmid of ~~Claims~~ Claim 2 wherein the naturally occurring 2µm-family plasmid is the 2µm plasmid as ~~obtained~~ from *Saccharomyces cerevisiae.*

Claim 6. (Currently amended) The 2µm-family plasmid of Claim 5 wherein the polynucleotide sequence insertion, deletion and/or substitution occurs at a position between ~~the~~ a first base of codon 59 of the *REP2* gene and the last base before the FRT site in the adjacent inverted repeat.

Claim 7. (Currently amended) The 2µm-family plasmid of Claim 5 wherein, other than the polynucleotide sequence insertion, deletion and/or substitution, the sequence of the *REP2* gene and the adjacent inverted repeat ~~is as defined by SEQ ID NO: 1 or variant thereof.~~comprises the nucleotides of SEQ ID NO: 1, or a nucleotide sequence 95% identical to SEQ ID NO:1.

Claim 8. (Currently amended) The 2µm-family plasmid of Claim 1 wherein polynucleotide sequence insertion, deletion and/or substitution occurs at a position between ~~the~~ a first base of the inverted repeat and ~~the~~ a last base before the FRT site.

Claim 9. (Currently amended) The 2µm-family plasmid of Claim 1 wherein the polynucleotide sequence insertion, deletion and/or substitution occurs between ~~the~~ a first base after the end of the *REP2* coding sequence and the last base before the FRT site~~, such as at the first base after the end of the *REP* 2 coding sequence~~.

Claim 10. (Currently amended) The 2µm-family plasmid of Claim 1wherein, other than the polynucleotide sequence insertion, deletion and/or substitution, the inverted repeat that follows the *REP2* coding sequence has a sequence ~~derived~~ from ~~the~~a corresponding region of the 2µm plasmid ~~as obtained~~ from *Saccharomyces cerevisiae* ~~and preferably the polynucleotide sequence insertion, deletion and/or substitution occurs at an *XcmI* site or an *FspI* site within the inverted repeat~~.

Claim 11. (Currently amended) The 2µm-family plasmid of Claim 5 wherein the polynucleotide sequence insertion, deletion and/or substitution occurs at a position between ~~the~~ a first base of codon 344 of the *FLP* gene and the last base before the FRT site in the adjacent inverted repeat.

Claim 12. (Currently amended) The 2µm-family plasmid of Claim 5 wherein, other than the polynucleotide sequence insertion, deletion and/or substitution, the sequence of the *FLP* coding sequence and the adjacent inverted repeat comprises the nucleotides of SEQ ID NO: 2, or a nucleotide sequence 95% identical to SEQ ID NO:2.~~is as defined by SEQ ID NO:2 or variant thereof.~~

Claim 13 . (Currently amended) The 2µm-family plasmid of Claim 11 wherein the polynucleotide sequence insertion, deletion and/or substitution occurs at a position between ~~the~~ a first base of the inverted repeat and the last base before the FRT site.

Claim 14. (Currently amended) The 2µm-family plasmid of Claim 13 wherein the polynucleotide sequence insertion, deletion and/or substitution occurs at a position between ~~the~~a first base after the end of the *FLP* coding sequence and the last base before the FRT site.

Claim 15. (Currently amended) The 2µm-family plasmid of Claim 14 wherein the polynucleotide sequence insertion, deletion and/or substitution occurs at ~~the~~ a first base after the end of the *FLP* coding sequence.

Claim 16. (Currently amended) The 2µm-family plasmid of Claim 11 wherein, other than the polynucleotide sequence insertion, deletion and/or substitution, the inverted repeat that follows the *FLP* gene has a sequence ~~derived~~ from ~~the~~ a corresponding region of the 2µm plasmid ~~as obtained~~ from *Saccharomyces cerevisiae*~~, and preferably the polynucleotide sequence insertion, deletion and/or substitution occurs at an *HgaI* site or an *FspI* site within the inverted repeat~~.

Claim 17. (Currently amended) The 2µm-family plasmid of Claim 1comprising polynucleotide sequence insertions, deletions and/or substitutions between ~~the~~ a first ~~bases~~ base after the last functional codons of both of the *REP2* gene and the *FLP* gene and a last ~~bases~~ base before the FRT sites in the inverted repeats adjacent to each of said genes, which polynucleotide sequence insertions, deletions and/or substitutions can be the same or different.

Claim 18. (Currently amended) The 2µm-family plasmid of Claim 1~~additionally~~, comprising a polynucleotide sequence insertion, deletion and/or substitution which is not ~~at a position as defined in any one of the preceding claims~~between the first base and the last base.

Claim 19. (Original) The 2µm-family plasmid of Claim 18 wherein the polynucleotide sequence insertion, deletion and/or substitution occurs within an untranscribed region around an ARS sequence.

Claim 20. (Previously presented) The 2μm-family plasmid of Claim 1 wherein the, or at least one, polynucleotide sequence insertion, deletion and/or substitution is a polynucleotide sequence insertion.

Claim 21. (Original) The 2μm-family plasmid of Claim 20 in which the polynucleotide sequence insertion encodes an open reading frame.

Claim 22. (Original) The 2μm-family plasmid of Claim 21 in which the open reading frame encodes a non-2μm-family plasmid protein.

Claim 23. (Currently amended) The 2μm-family plasmid of Claim 22 in which the non-2μm-family plasmid protein comprises the sequence of a protein involved in protein folding, or which has chaperone activity or is involved in the unfolded protein response, albumin, a monoclonal antibody, an etoposide, a serum protein ~~(such as a blood clotting factor)~~, antistasin, a tick anticoagulant peptide, transferrin, lactoferrin, endostatin, angiostatin, collagens, immunoglobulins or immunoglobulin-based molecules or fragments of either (e.g. a dAb, Fab' fragments, F(ab')2, scAb, scFv or scFv fragment), a Kunitz domain protein, interferons, interleukins, IL 10, IL 11, IL2, interferon α species and sub-species, interferon β species and sub-species, interferon γ species and subspecies, leptin, CNTF, $CNTF_{Ax15}$, IL 1-receptor antagonist, erythropoietin (EPO) and EPO mimics, thrombopoietin (TPO) and TPO mimics, prosaptide, cyanovirin-N, 5-helix, T20 peptide, T1249 peptide, HIV gp41, HIV gp120, urokinase, prourokinase, tPA, hirudin, platelet derived growth factor, parathyroid hormone, proinsulin, insulin, glucagon, glucagon-like peptides, insulin-like growth factor, calcitonin, growth hormone, transforming growth factor β, tumour necrosis factor, G-CSF, GM-CSF, M-CSF, FGF, coagulation factors in both pre and active forms, including but not limited to plasminogen, fibrinogen, thrombin, pre-thrombin, pro-thrombin, von Willebrand's factor, ~~α1-antitrypsin~~ α1-antitrypsin, plasminogen activators, Factor VII, Factor VIII, Factor IX, Factor X and Factor XIII, nerve growth factor, LACI, platelet-derived endothelial cell growth factor (PD-ECGF), glucose oxidase, serum cholinesterase, aprotinin, amyloid precursor protein, inter-alpha trypsin inhibitor, antithrombin III, apo-lipoprotein species, Protein C, or Protein S~~, or a variant or fragment of any of the above.~~.

Claim 24. (Currently amended) The 2µm-family plasmid of Claim 23 in which the 2µm-family plasmid protein comprises the sequence of albumin~~, a variant or fragment thereof, or a fusion protein comprising the sequence of any of these.~~.

Claim 25. (Currently amended) The 2µm-family plasmid of Claim 23 in which the non-2µm-family plasmid protein comprises the sequence of transferrin~~, a variant or fragment thereof, or a fusion protein comprising the sequence of any of these.~~.

Claim 26. (Currently amended) The 2µm-family plasmid of Claim 23 in which the non-2µm-family plasmid protein comprises the sequence of lactoferrin~~, a variant or fragment thereof, or a fusion protein comprising the sequence of any of these.~~.

Claim 27. (Currently amended) The 2µm-family plasmid of Claim 23 in which the non-2µm-family plasmid protein comprises the sequence of Fc~~, a variant or fragment thereof, or a fusion protein comprising the sequence of any of these.~~.

Claim 28. (Original) The 2µm-family plasmid of Claim 23 in which the non-2µm-family plasmid protein comprises the sequence of a protein involved in protein folding, or which has chaperone activity or is involved in the unfolded protein response as encoded by anyone of *AHA1, CCT2, CCT3, CCT4, CCT5, CCT6, CCT7, CCT8, CNS1, CPR3,CPR6, EPS1, ERO1, EUG1, FMO1 HCH1, HSP10, HSP12, SP104,HSP26,HSP30,HSP42,HSP60,HSP78,HSP82,JEM1,MDJ1, MDJ2, MPD1, MPD2,PD11,PFD1, ABC1, APJ1, ATP11, ATP12, BTT1, CDC37, CPR7, HSC82, KAR2, LHS1, MGE1, MRS11, NOB1, ECM10,SSA1,SSA2,SSA3,SSA4,SSC1,SSE2,SIL1,SLS1, UBI4,ORM1,ORM2, PER1, PTC2, PSE1* and *HAC1* or a truncated intronless *HAC1*.

Claim 29. (Previously presented) The 2µm-family plasmid of Claim 23 in which the chaperone is protein disulphide isomerase (PDI), or is a protein encoded by *ORM2, SSA1* or *PSE1*.

Claim 30. (Previously presented) The 2µm-family plasmid of Claim 22 in which the non-2µm-family plasmid protein comprises a secretion leader sequence.

Claim 31. (Original) The 2µm-family plasmid of Claim 22 in which the non-2µm-family plasmid protein comprises the sequence of a bacterial selectable marker and/or a yeast selectable marker.

Claim 32. (Original) The 2μm-family plasmid of Claim 31 in which the bacterial selectable marker is a β-lactamase gene and/or the yeast selectable marker is a *LEU2* selectable marker.

Claim 33. (Currently amended) The 2μm-family plasmid according to Claim 1, which plasmid comprises (i) a heterologous sequence encoding a non- 2μm-family plasmid protein; (ii) a heterologous sequence encoding a protein comprising the sequence of a protein involved in protein folding, a chaperone or a protein involved in the unfolded protein response~~, preferably protein disulphide isomerase~~; and (iii) a heterologous sequence encoding a protein comprising the sequence of a selectable marker; wherein at least one of the heterologous sequences occurs ~~at a position as defined by any one of Claims 1 to 16.~~ between the first base after the last functional codon of at least one of either the *REP2* gene or the *FLP* gene and the last base before the FRT site in an inverted repeat adjacent to the gene.

Claim 34. (Currently amended) A method of preparing a plasmid as defined by Claim 1 comprising:

(a) providing a plasmid comprising the sequence of a *REP* 2 gene and the inverted repeat that follows the *REP2* gene, or a *FLP* gene and the inverted repeat that follows the *FLP* gene, in each case the inverted repeat comprising an FRT site;

(b) providing a polynucleotide sequence and inserting the polynucleotide sequence into the plasmid ~~at a position as defined in~~of Claim 1 between the first base after the last functional codon of at least one of either the *REP2* gene or the *FLP* gene and the last base before the *FRT* site in an inverted repeat adjacent to the gene; and/or

(c) deleting some or all of the nucleotide bases ~~at the positions~~ between the first base after the last functional codon of at least one of either the *REP2* gene or the *FLP* gene and the last base before the FRT site in an inverted repeat adjacent to the gene of ~~defined in~~ Claim 1; and/or

(d) substituting some or all of the nucleotide bases between the first base after the last functional codon of at least one of either the *REP2* gene or the *FLP* gene and the last base before the FRT site in an inverted repeat adjacent to the gene ~~at the positions defined in Claim 1~~ with alternative nucleotide bases.

Claim 35. (Original) A plasmid obtainable by the method of Claim 34.

Claim 36. (Previously presented) A host cell comprising a plasmid as defined by Claim 1.

Claim 37. (Original) A host cell according to Claim 36 which is a yeast cell.

Claim 38. (Previously presented) A host cell according to Claim 36 in which the plasmid is stable as a multicopy plasmid.

Claim 39. (Currently amended) A host cell according to Claim 38 in which the plasmid comprises a polynucleotide sequence insertion, deletion and/or substitution between a first base after a last functional codon of at least one of either an *REP* 2 gene or an *FLP* gene and a last base before an FRT site in an inverted repeat adjacent to said gene.~~is based on pSR1, pSB3 or pSB4 and the yeast cell is *Zygosaccharomyces rouxii*, the plasmid is based on pSB1 or pSB2 and the yeast cell is *Zygosaccharomyces bailli*, the plasmid is based on pSM1 and the yeast cell is *Zygosaccharomyces fermentati*, the plasmid is based on pKD1 and the yeast cell is *Kluyveromyces drosophilarum*, the plasmid is based on pPM1 and the yeast cell is *Pichia membranaefacions* or the plasmid is based on the 2µm plasmid and the yeast cell is *Saccharomyces cerevisiae* or *Saccharomyces carlsbergensis*.~~

Claim 40. (Currently amended) A host cell according to Claim 38 in which, if the plasmid contains, or is modified to contain, a selectable marker then stability, as measured by the loss of the marker, is at least 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.9% or ~~substantially~~ 100% after 5 generations.

Claim 41. (Previously presented) A method of producing a protein comprising the steps of-

(a) providing a plasmid as defined by Claim 1;

(b) providing a suitable host cell;

(c) transforming the host cell with the plasmid; and

(d) culturing the transformed host cell in a culture medium;

(e) thereby to produce the protein.

Claim 42. (Currently amended) A method of producing a protein comprising the steps of providing a host cell as defined by Claim 36 which host cell comprises a plasmid comprising a polynucleotide sequence insertion, deletion and/or substitution between the first base after the last functional codon of at least one of either a *REP* 2 gene or an *FLP* gene and the last base before the FRT site in an inverted repeat adjacent to said gene as ~~defined by Claim 1~~ and culturing the host cell in a culture medium thereby to produce the protein.

Claim 43. (Previously presented) A method according to Claim 41 further comprising the step of isolating the thus produced protein from the cultured host cell or the culture medium.

Claim 44. (Currently amended) A method according to Claim 43 further comprising the step of purifying the thus isolated protein ~~to a commercially acceptable level of purity~~.

Claim 45. (Original) A method according to Claim 44 further comprising the step of formulating the thus purified protein with a carrier or diluent, and optionally presenting the thus formulated protein in a unit form.

Claim 46. (Canceled)

Claim 47. (Currently amended) A method according to Claim 44 further comprising the step of formulating the ~~thus~~ purified protein with a pharmaceutically acceptable carrier or diluent and optionally presenting the thus formulated protein in a unit dosage form.

Claims 48 - 63. (Canceled).

Claim 64 (New) The 2µm-family plasmid of Claim 11, wherein the polynucleotide sequence insertion, deletion and/or substitution occurs at an *HgaI* site or an *FspI* site within the inverted repeat.

Claim 65. (New) The 2µm-family plasmid of Claim 1, wherein the plasmid comprises a heterologous sequence encoding protein disulphide isomerase.

Claim 66 (New) The 2μm-family plasmid of Claim 1, wherein the plasmid comprises a heterologous sequence encoding a protein of interest.